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Characterisation of Collagen Scaffolds using X-ray Microtomography

Patrick J. Smith, Eleftherios Sachlos[1], Samuel McDonald, Nuno Reis, Brian Derby, Paul M. Mummery and Jan T. Czernuszka[1]
Manchester Materials Science Centre, UMIST and University of Manchester, Grosvenor Street, Manchester, M1 7HS, England.
[1] Department of Materials, University of Oxford, Parks Road, Oxford, OX1 3PH, England.

ABSTRACT

Collagen scaffolds have been produced that incorporate predefined internal channels. The scaffolds were obtained with the aid of sacrificial moulds that have been manufactured using a rapid prototyping technique. A computer aided design file of the mould was created and then realised using an ink-jet printer. A dispersion of collagen was then cast into the mould and frozen. Ethanol was used to dissolve the mould leaving the collagen, which was then freeze dried to produce the final product. The scaffold was then analysed using X-ray microtomography (XMT) to determine whether the desired internal structure was obtained. It was found necessary to add saturated potassium iodide (KI) solution to the scaffold in order to analyse it satisfactorily by XMT. The resultant images indicate that the desired internal structure was obtained.

INTRODUCTION

The new multidisciplinary field of Tissue Engineering has arisen to satisfy the demand for biological substitutes to repair living tissue [1]. Tissue engineering involves the growth of relevant biological material into the required organ or tissue. However, unaided cells do not grow into the required orientations and therefore the resulting tissues are not of the correct shape. A solution is provided by the use of three-dimensional (3D) scaffolds acting as guides for cellular growth. Tissue engineered scaffolds are porous structures usually made from bioresorbable material containing appropriate factors to induce cell adhesion and growth. This allows the attached cells to migrate and colonise the whole scaffold. During the scaffold degradation, cells proliferate and occupy newly liberated spaces to create a viable tissue replacement [2].

Biodegradable and bioresorbable polymers and ceramics have been used to make 3D scaffolds [3 - 5]. Both synthetic and natural polymers have been used. The material used in this investigation is the natural polymer, collagen. Collagen is an abundant protein present in the connective tissue (and extra-cellular matrix in bone) of mammals.

Existing methods for scaffold fabrication are dependant on the generation of pores within the matrix; typical pore generators are salt particles and ice crystals. The distribution of pores however cannot be controlled precisely. As a consequence, current techniques cannot produce complicated internal features, e.g. channels that could act as an artificial vascular system. This internal system is desirable because it allows the flow of a blood-like medium throughout the construct, which supplies oxygen and nutrients to cells migrating deep into the scaffold.

Solid Freeform Fabrication (SFF), an outgrowth of Rapid Prototyping (RP), can have a great impact on tissue engineering by producing scaffolds with tailored architectures. SFF processes can deliver complex shapes with intricate internal features directly from computer-generated models [6]. In this paper, an inkjet printing technique has been used to produce a 3D

mould, which is the negative shape of the desired scaffold. The collagen scaffolds were then made by freezing an aqueous dispersion of collagen inside the sacrificial mould. This results in the nucleation of ice crystals, which grow and force the collagen into the interstitial spaces, thereby aggregating the collagen. The ice crystals are removed by sublimation using a conventional freeze-drying technique.

As-produced collagen scaffolds have been analysed using X-ray microtomography [7-9]. This technique is favourable as it is non-destructive and also enables 3D analysis from a set of cross-sections. Techniques such as scanning electron microscopy and mercury porosimetry are destructive and only provide two-dimensional information that may not be representative of the whole construct. This paper reports the results of using X-ray microtomography to analyse 3D collagen scaffolds.

EXPERIMENTAL

The preparation of the collagen scaffolds used in this study is described in greater detail elsewhere [2]. Briefly, moulds with the required negative-shape, were designed using commercial CAD software and manufactured using the commercial SFF system, Model-Maker II (Solidscape Inc. Merrimack, NH, USA). Figures 1 and 2 show the mould design used to make the collagen scaffolds and relevant cross-sectional areas of the mould respectively.

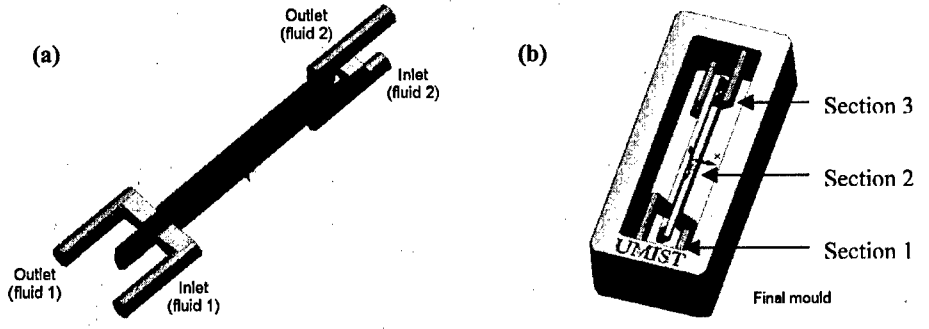

Figure 1. The mould design used to make the collagen scaffolds, (a) shows the internal channel structure and (b) the final mould indicating cross-sections of interest; these are shown in diagrammatical form in Figure 2.

The build material used in the mould was a proprietary low molecular weight polar material (ProtoBuild, Solidscape Inc.). A collagen dispersion was then cast into the mould and frozen at about –20 °C. The mould was then immersed in ethanol, which dissolved the mould and the ice crystals. The collagen scaffold was then dried after solvent exchange with liquid carbon dioxide. Figure 3 shows a typical mould and the collagen scaffold produced. Subsequent moulds produced for this study were doped with KI and methylene blue dye. The collagen scaffolds were also doped with an aqueous saturated potassium iodide (KI) solution.

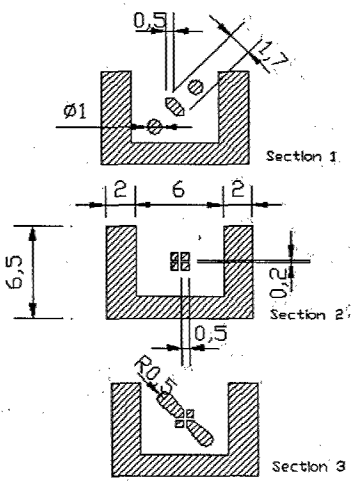

Figure 2. Cross sections of the mould design, which provides details of the desired internal structure. (All dimensions are in millimetres.)

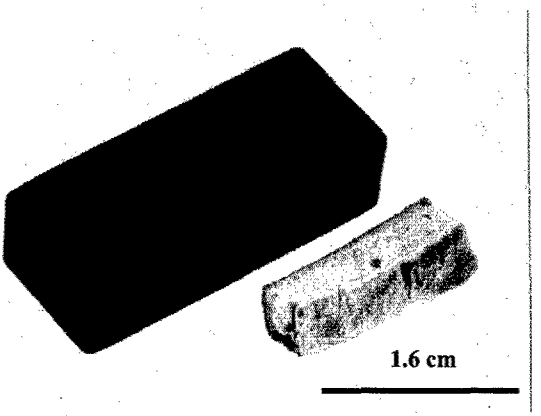

Figure 3. A typical Model Maker II produced mould next to a typical collagen scaffold.

The X-ray microtomographic system (XMT) used in this study was a HMX-225 radiography platform (X-Tek, Tring, UK) run under Tomahawk tomography software (AEA Technology, Harwell, UK). The XMT works by placing a specimen in the path of X-rays. The specimen can be rotated and also translated perpendicularly to the X-ray beam. In tomographic imaging, a number of absorption profiles of the specimen are captured using a cone projection for a complete rotation of the sample. Typically, a radiograph is taken every 0.5°. From these data, the 3D image can be reconstructed using a standard filtered back-projection algorithm [7]. The system allows the X-ray voltage and current to be varied independently.

RESULTS AND DISCUSSION

Initial observations of collagen scaffolds using XMT were disappointing due to poor contrast because of collagen's low X-ray absorption. Subsequently a doping regime was employed to enable the scaffolds to be detected using XMT. This involved applying a few drops of a saturated KI solution onto a collagen scaffold, which caused the scaffold to reduce in volume by approximately 50%. The necessary addition of the KI solution introduced surface tension forces that caused the collapse of the fragile pore structure. Figure 4 shows the images of slices taken in the *xy* direction of the KI doped collagen scaffold. When these two images are compared with the diagram in Figure 2 it can be clearly seen that Figure 4a corresponds with Section 3 in the diagram and Figure 4b corresponds with Section 1. That is to say that the scaffold has the desired internal structure.

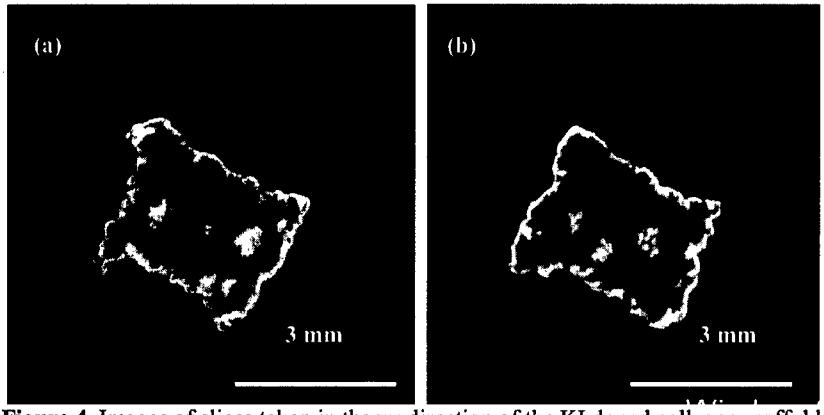

Figure 4. Images of slices taken in the xy direction of the KI doped collagen scaffold; (a) corresponds to section 3 and (b) corresponds to section 1 in Figure 1 respectively.

These images are encouraging; however further examination of Figure 4a and the design for Section 3 in Figure 2 shows a small mismatch. Section 3 consists of four discrete channels, whereas Figure 4a suggests that the channels have collapsed in on one another. It is thought that this apparent "merger" of channels is an effect of the KI doping. In the preparation of the collagen scaffolds great care is taken to protect the scaffolds from water. This is because the surface tension of the water would cause the collapse of the delicate porous structure [2]. However, in order to obtain XMT images it was necessary to add an aqueous solution of KI. Therefore, the above images actually show the dispersal of KI throughout the collagen matrix. Looking again at the diagram of Section 3 in Figure 2, it can be seen that the collagen separating the four chambers is very thin. It is thought that this area of the matrix has not absorbed any KI and therefore has not been detected by XMT. This is coupled with the collapse of porosity and the reduction in volume of the collagen scaffold. Figure 5 shows images taken in the *xy* direction of the scaffold corresponding to Section 2.

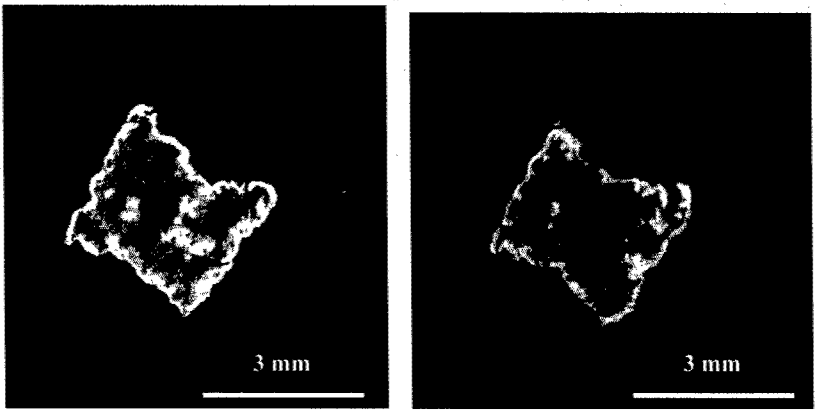

Figure 5. Images of slices taken in the *xy* direction of the KI doped collagen scaffold corresponding to section 2 in Figure 2.

It can be seen that the internal structure observed is a reasonable approximation to that expected. There is a lack of segregation between the four channels observed and it is thought this is due to the reasons stated.

Figure 6 show images of slices taken in the *xz* plane. It can be clearly seen that the desired internal structure has been obtained.

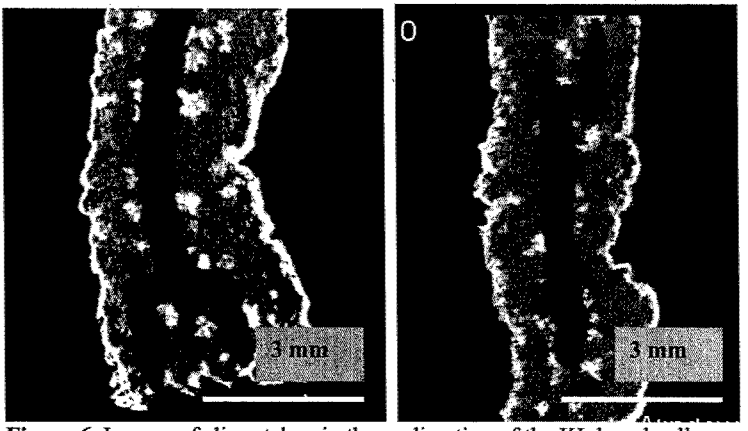

Figure 6. Images of slices taken in the *xz* direction of the KI doped collagen scaffold corresponding to section 2 in Figure 1.

This analysis shows that the collagen scaffolds do have the internal structure that has been designed for them. However, the necessary addition of KI solution has made those scaffolds that were analysed unusable. Various dopants were added in the preparation stage in order to remove the destructive step of aqueous KI solution addition, including KI itself. However, none of these dopants were detected by XMT.

CONCLUSION

Collagen scaffolds have been prepared using SFF produced moulds. The moulds incorporated a complex internal network of channels, which are necessary to the final function of the collagen structure. The scaffolds were analysed using XMT to determine whether the desired internal structure had been produced. Initially the scaffolds could not be imaged using XMT. This necessitated the addition of saturated KI solution. This enabled the scaffolds to be imaged. It could clearly be seen that the collagen scaffold has the internal structure that was designed.

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